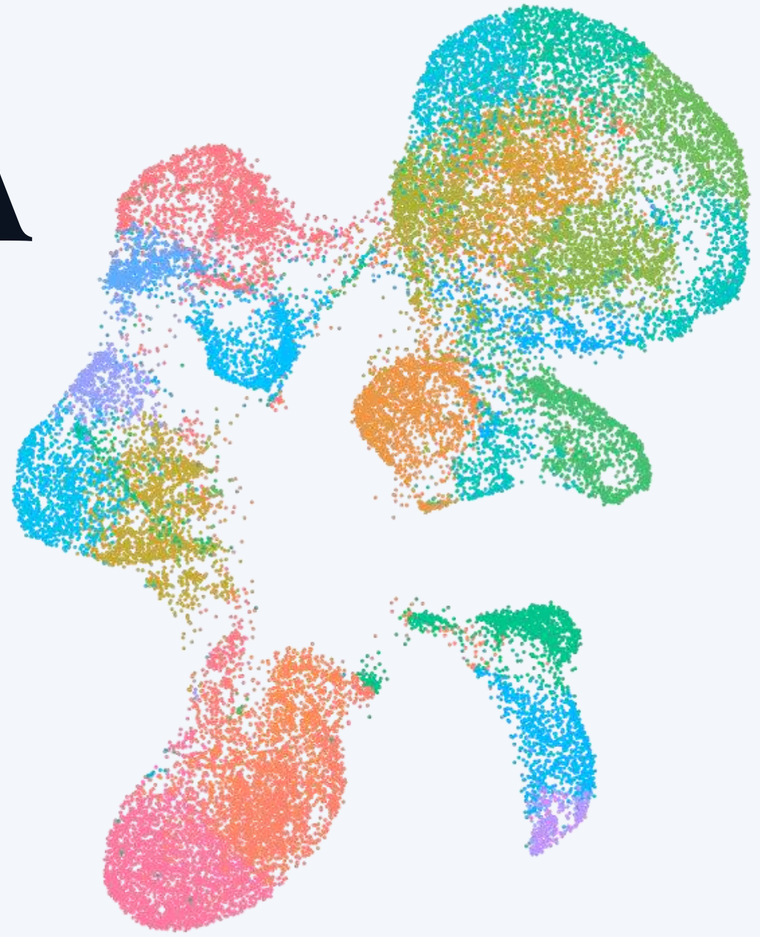
Single-cell RNA sequencing



Beatriz Martín Mur
Data Analyst – Functional Genomics Team
Centro Nacional de Análisis Genómico (CNAG)

CNAG

SEQUENCING CAPACITY

>10,000 Gbases/day = 100 human genomes/day at 30x

SEQUENCING INSTRUMENTS

5 Illumina sequencers (3 NovaSeq6000, 1 HiSeq4000, 1 MiSeq)

2 Oxford Nanopore Technologies sequencers (1 Gridlon, 1 Promethion)

SINGLE-CELL GENOMICS

10x Chromium Controller

10x Chromium Connect

SPATIAL GENOMICS

Bruker Vutara microscope

Nanostring CosMX

COMPUTING CAPACITY

10,000 cores

14 PB disk + 7 PB tape

Functional Genomics

Bulk RNA-seq

- Differential gene expression
- Differential alternative splicing
- Gene fusions detection
- SNP calling

Small RNA-seq

- Differential miRNA expression
- miRNA annotation

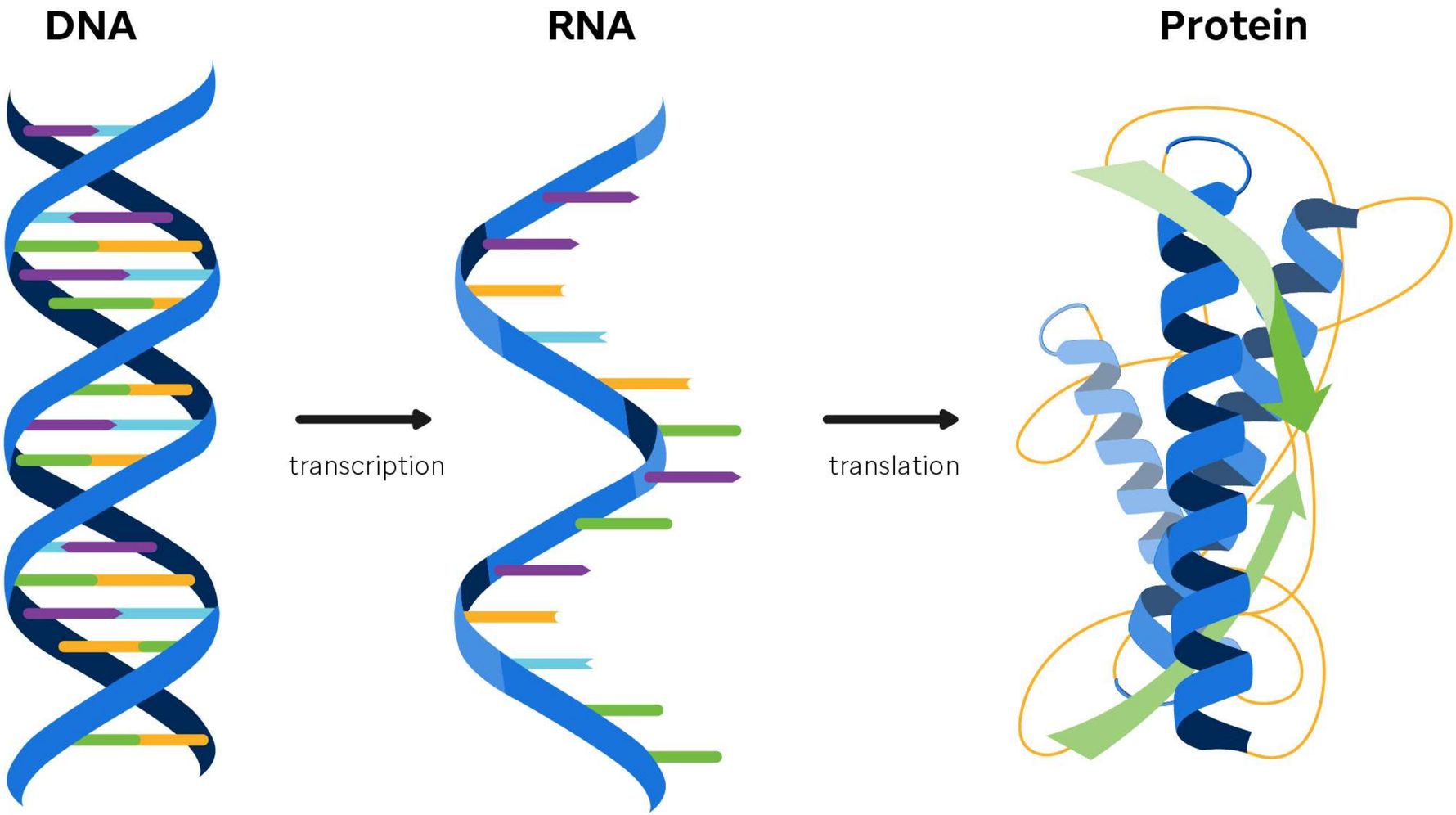
Single-cell RNA-seq

- Annotation of cell types
- Cell type differential expression
- Trajectory inference
- BCR/TCR profiling
- Differential accessibility regions

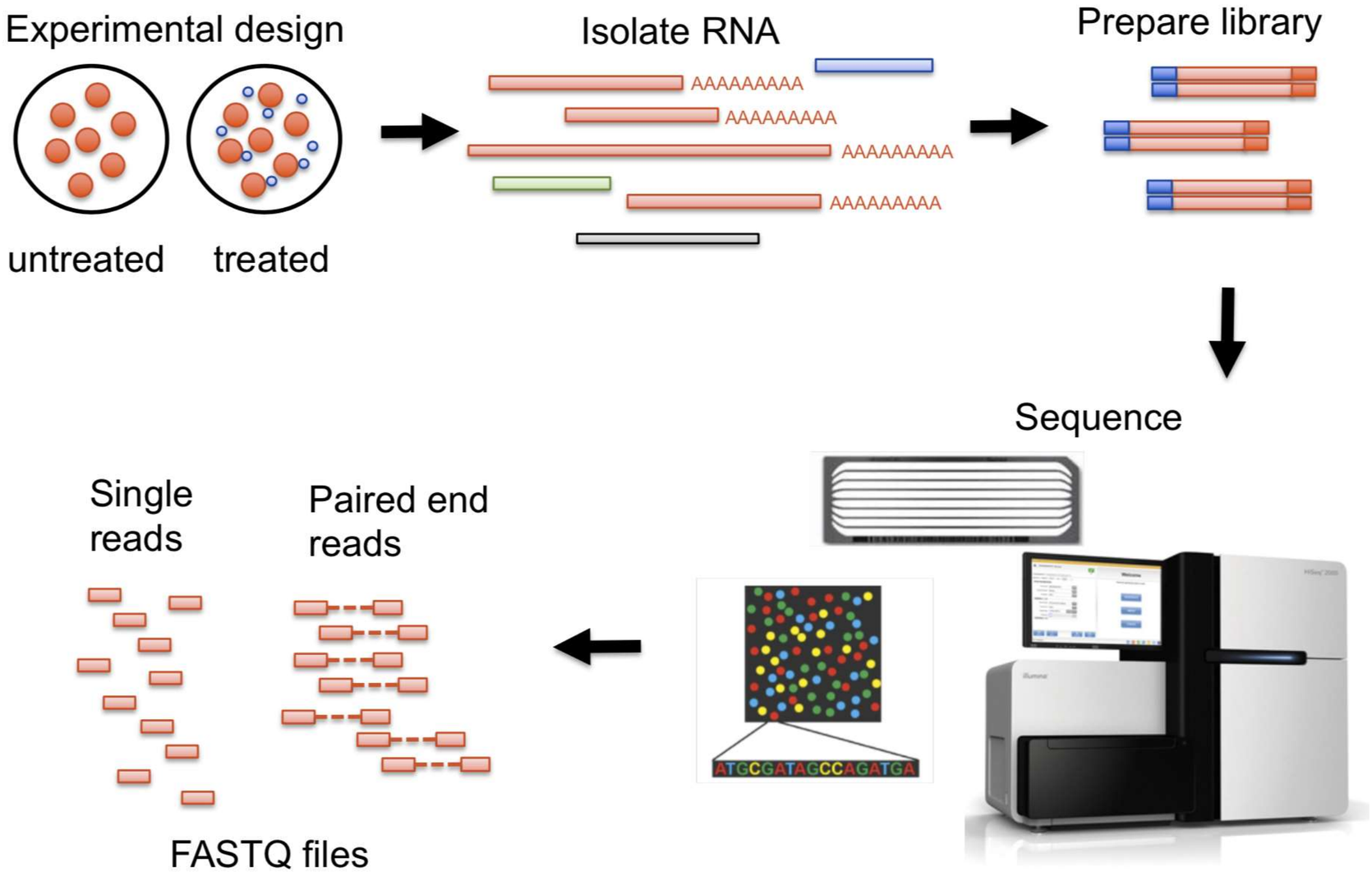
Long read RNA (ONT)

- Quantification of annotated and novel isoforms
- Differential isoform expression
- Differential transcript usage
- RNA modifications
- Gene fusion detection

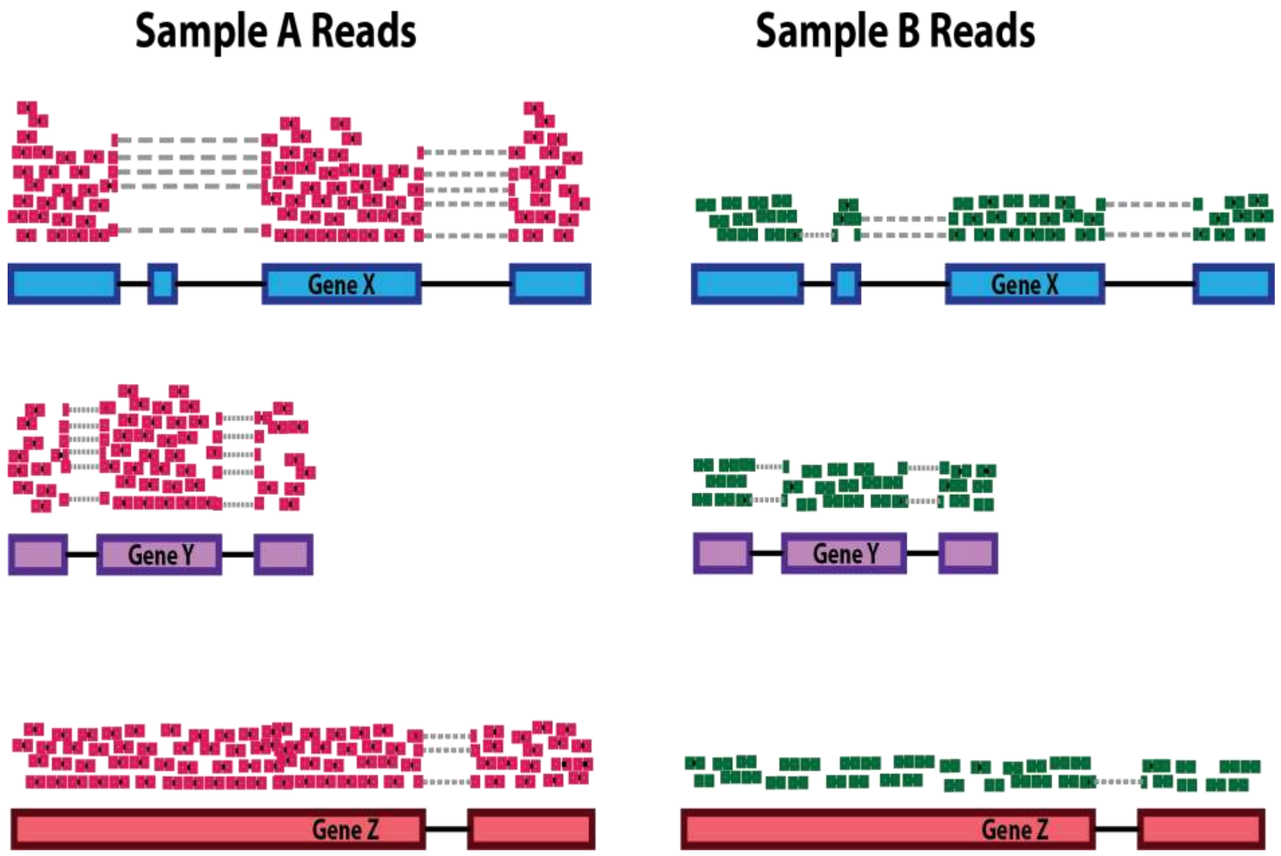
Central Dogma



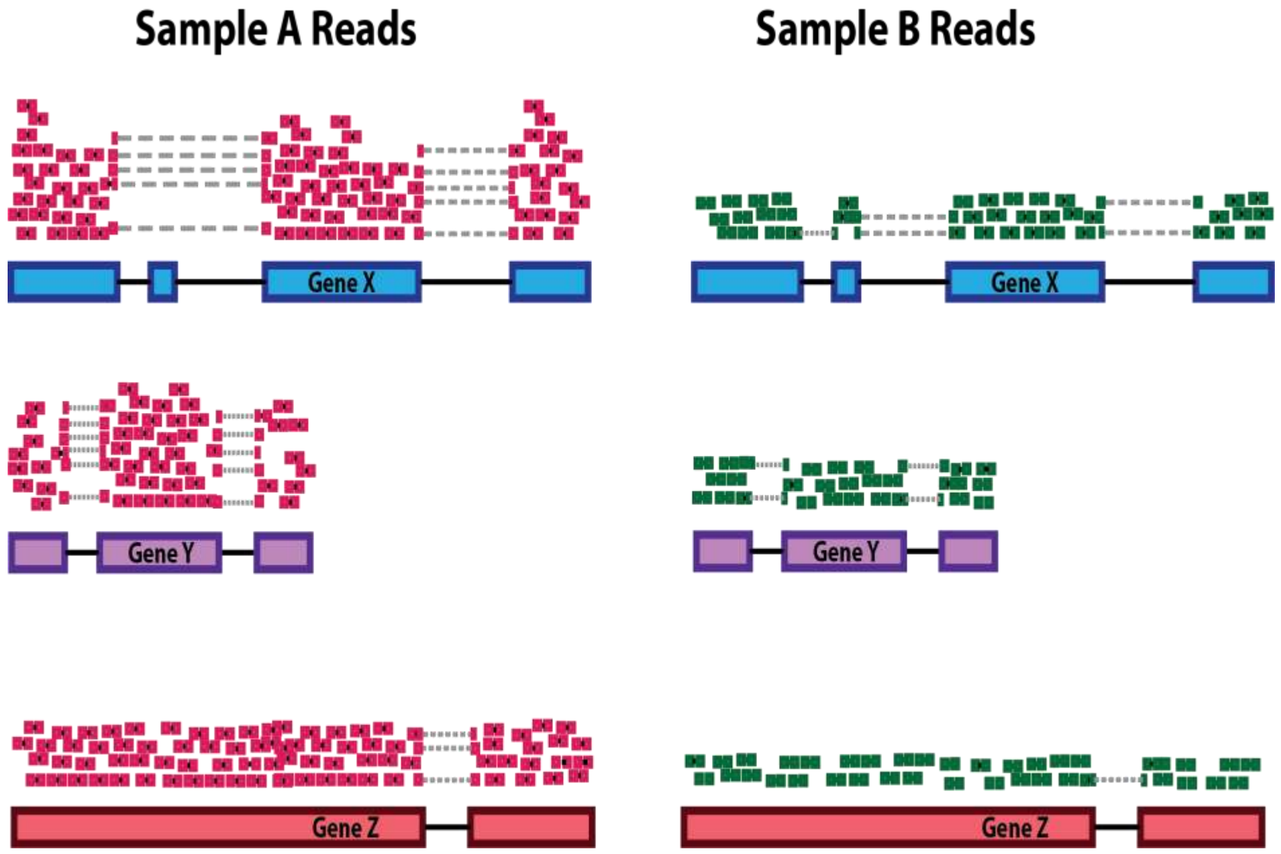
Differential expression

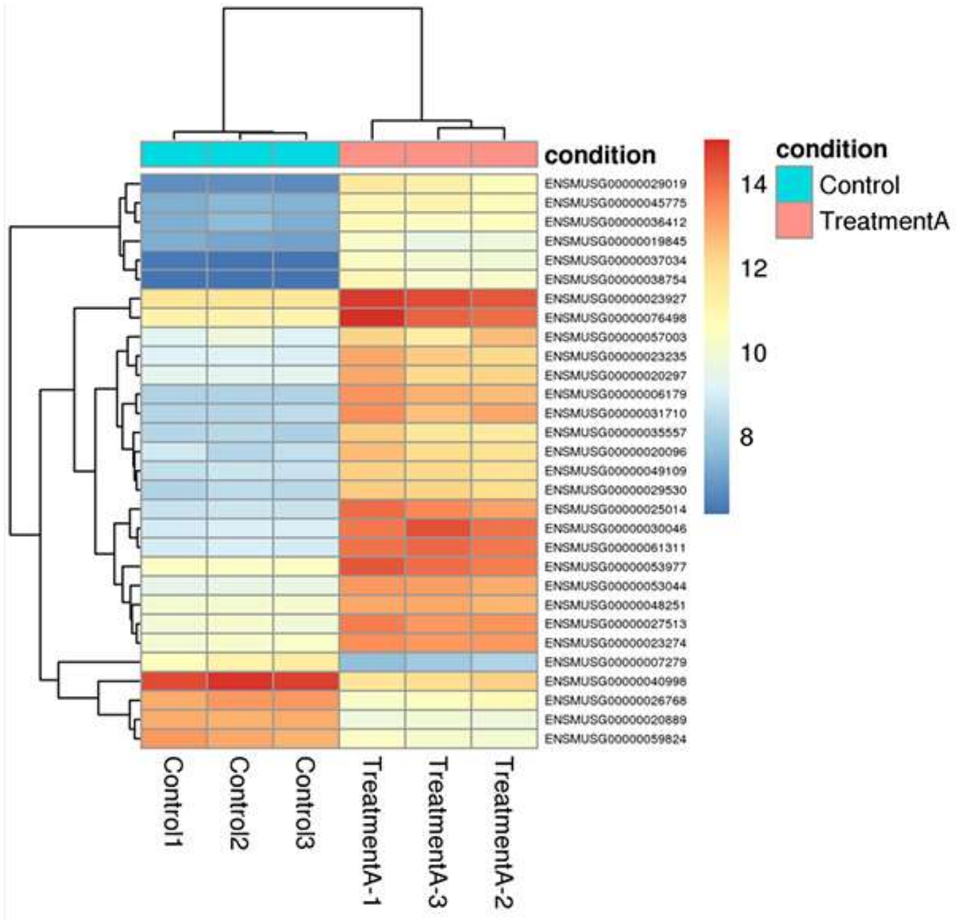


Differential expression

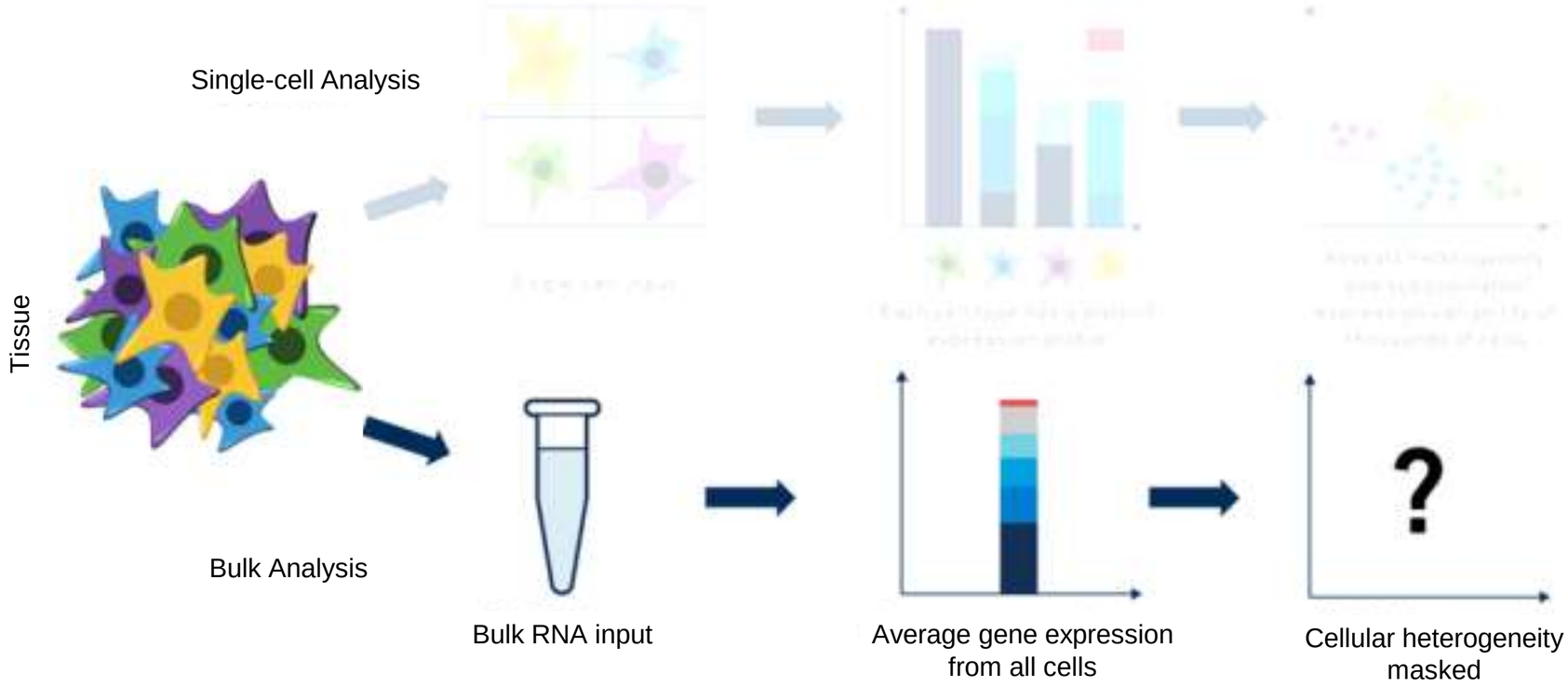


Differential expression





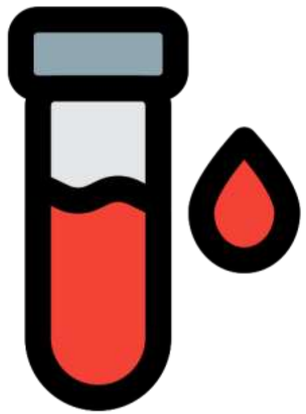
Bulk RNA-seq

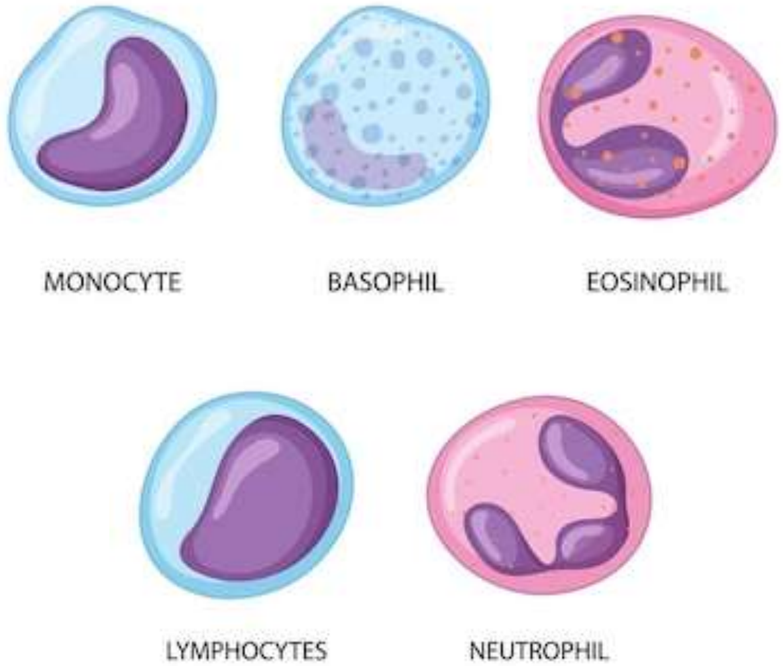

Single-cell Analysis
Tissue
Bulk Analysis
Bulk RNA input
Average gene expression from all cells
?
Cellular heterogeneity masked

Bulk vs single-cell

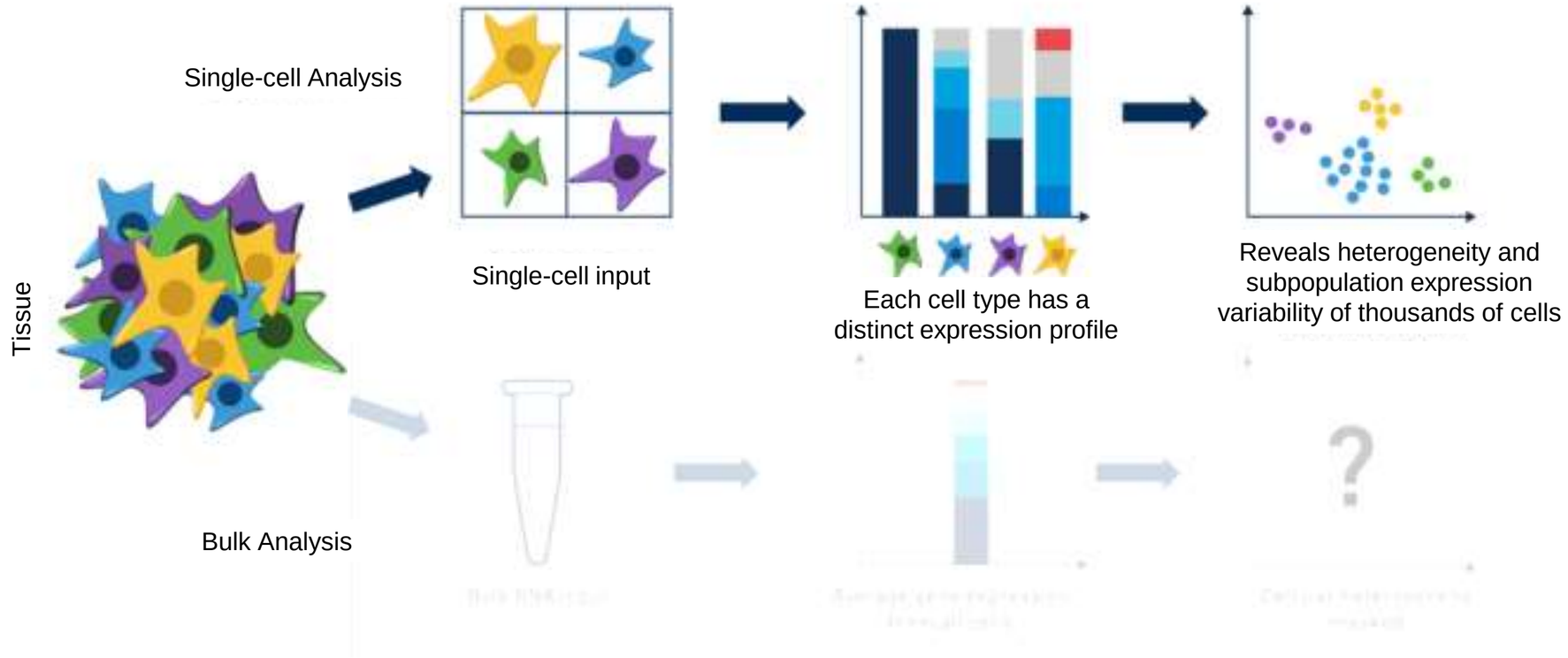
Bulk vs single-cell

Bulk vs single-cell

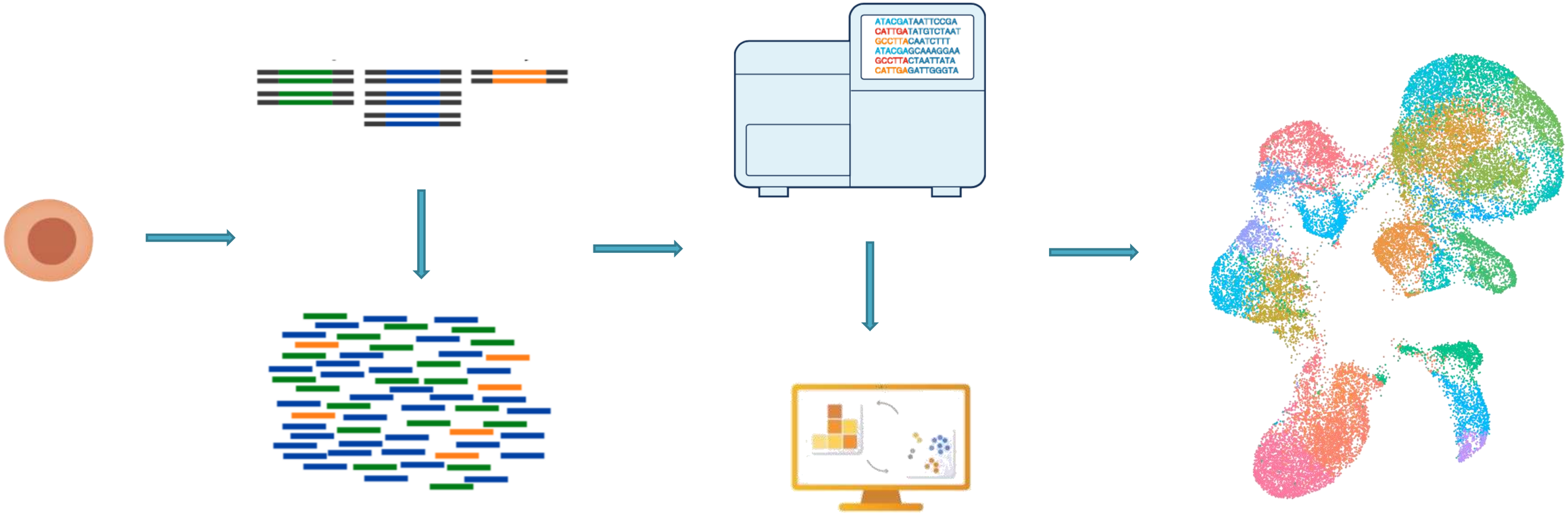




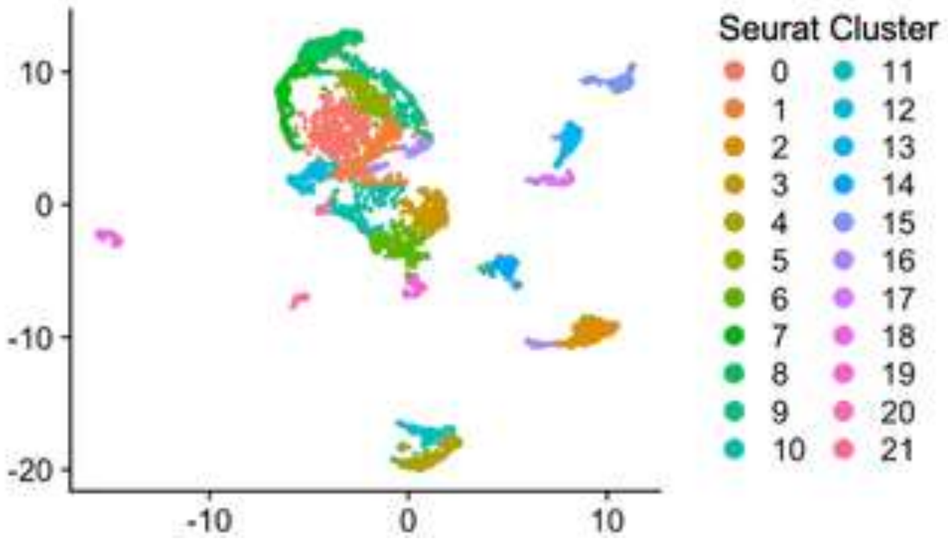
Single-cell RNA-seq



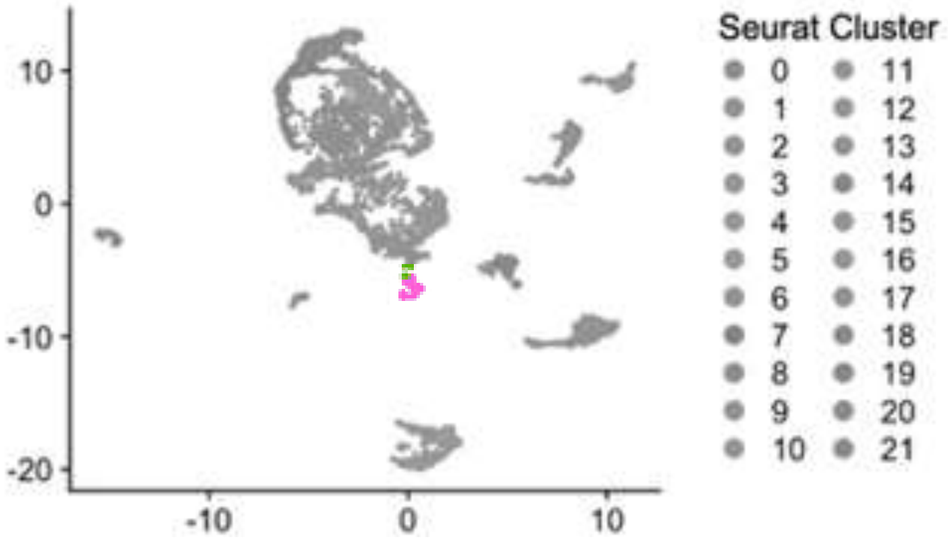
Single-cell RNA-seq



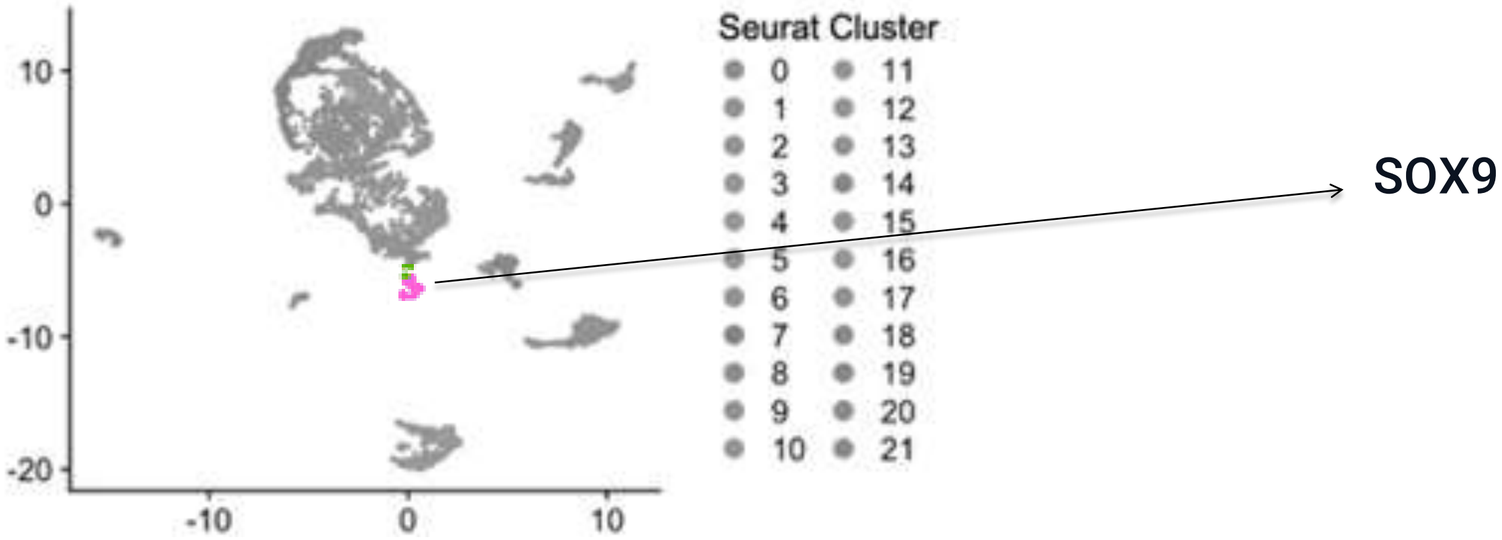
Single-cell RNA-seq



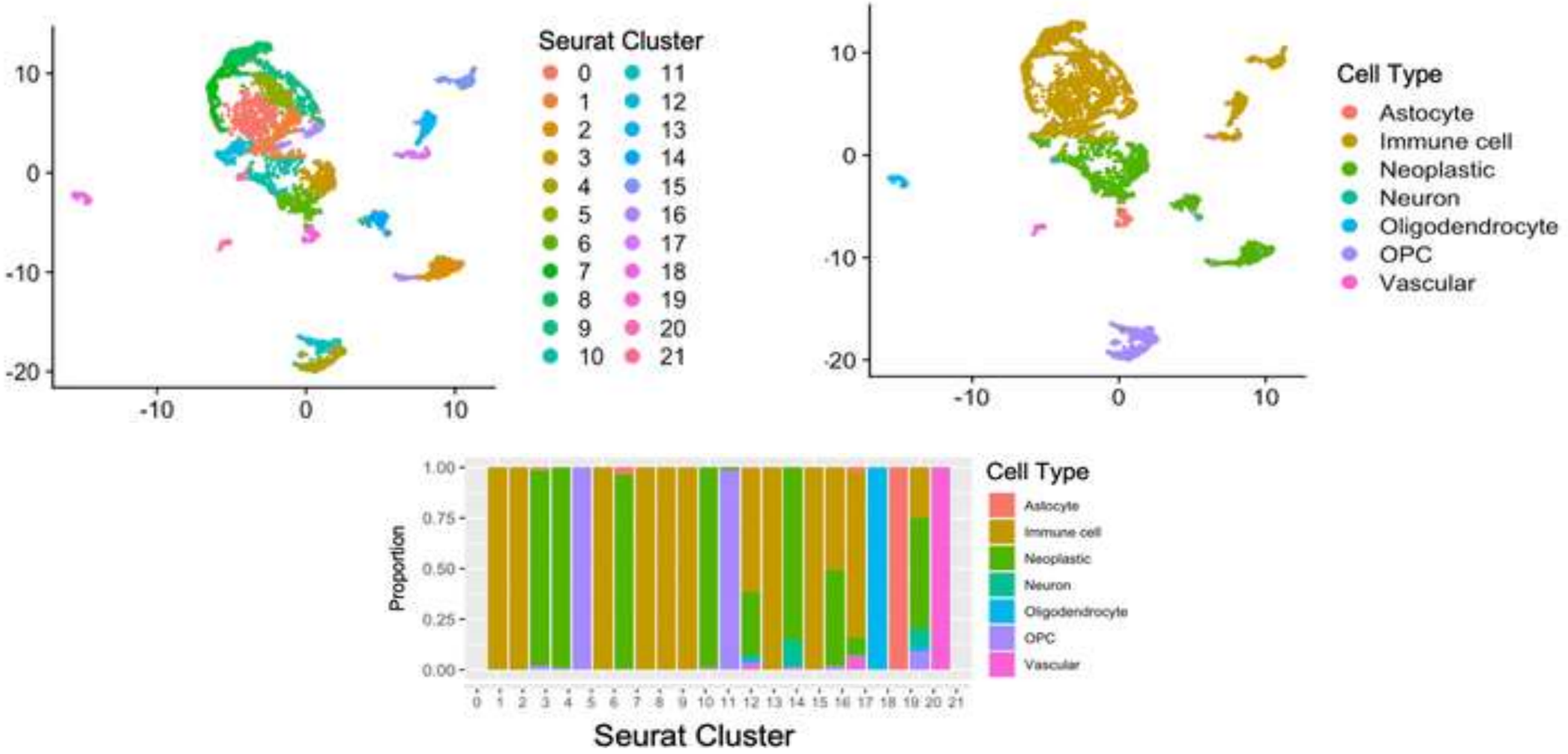
Single-cell RNA-seq



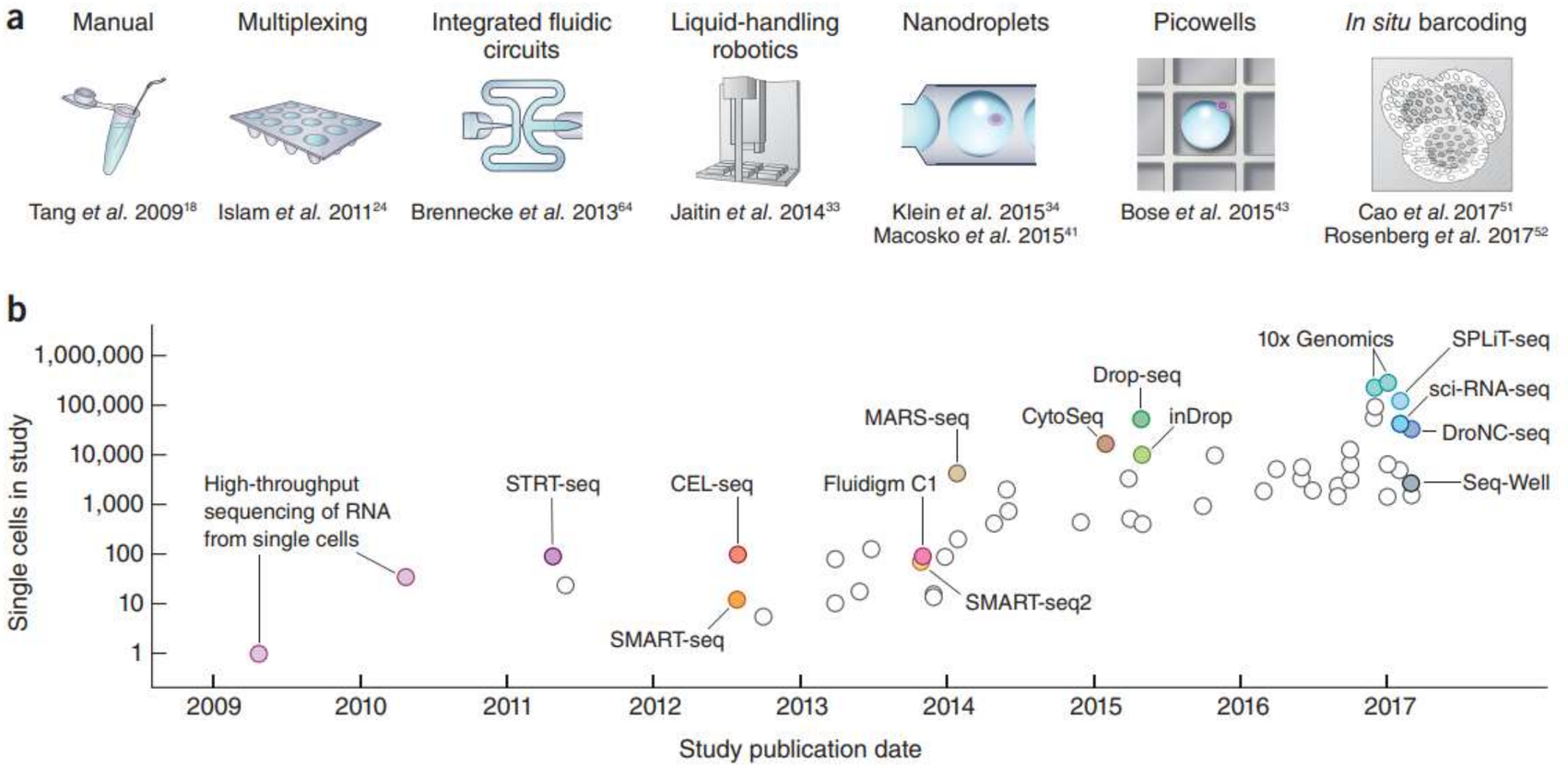
Single-cell RNA-seq



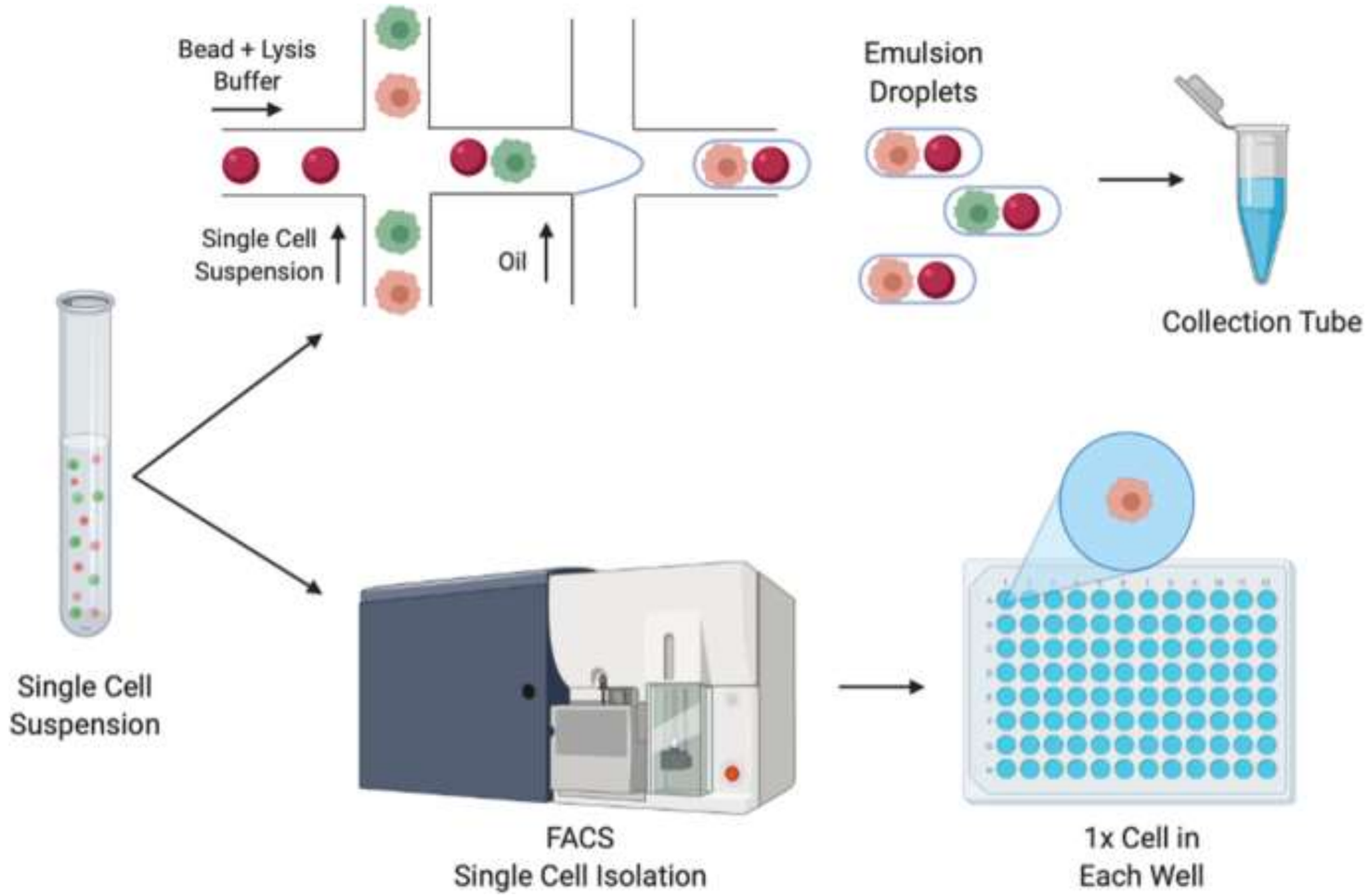
Single-cell RNA-seq



Protocols


a
Manual
Multiplexing
Integrated fluidic circuits
Liquid-handling robotics
Nanodroplets
Picowells
In situ barcoding
Tang *et al.* 2009[18]
Islam *et al.* 2011[24]
Brennecke *et al.* 2013[64]
Jaitin *et al.* 2014[33]
Klein *et al.* 2015[34]
Macosko *et al.* 2015[41]
Bose *et al.* 2015[43]
Cao *et al.* 2017[51]
Rosenberg *et al.* 2017[52]
b
Single cells in study
1,000,000
100,000
10,000
1,000
100
10
1
2009
2010
2011
2012
2013
2014
2015
2016
2017
Study publication date
High-throughput sequencing of RNA from single cells
STRT-seq
CEL-seq
SMART-seq
MARS-seq
Fluidigm C1
SMART-seq2
CytoSeq
Drop-seq
inDrop
10x Genomics
SPLiT-seq
sci-RNA-seq
DroNC-seq
Seq-Well

Protocols


Bead + Lysis Buffer
Single Cell Suspension
Oil
Emulsion Droplets
Collection Tube
Single Cell Suspension
FACS Single Cell Isolation
1x Cell in Each Well

Protocols

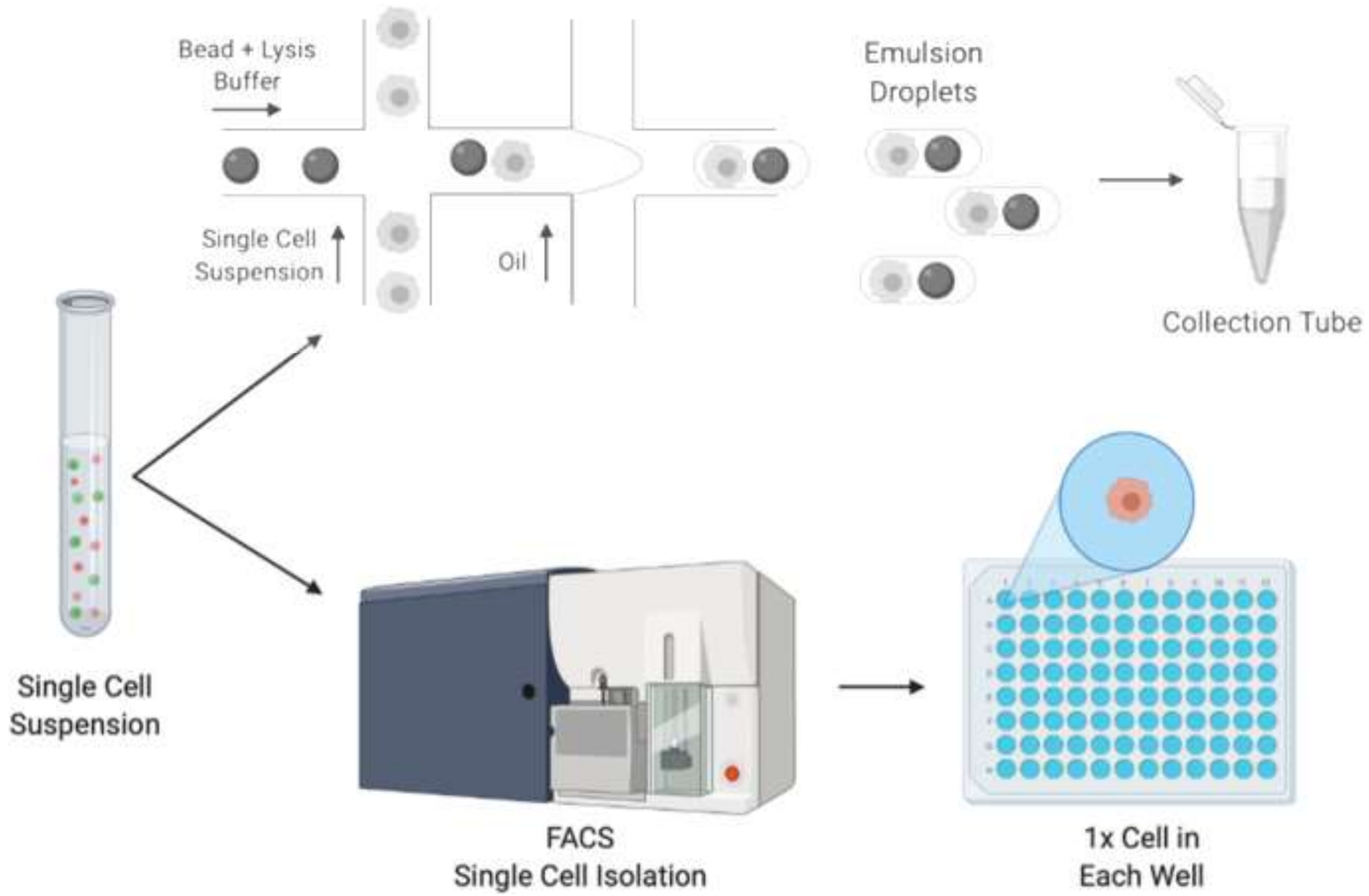
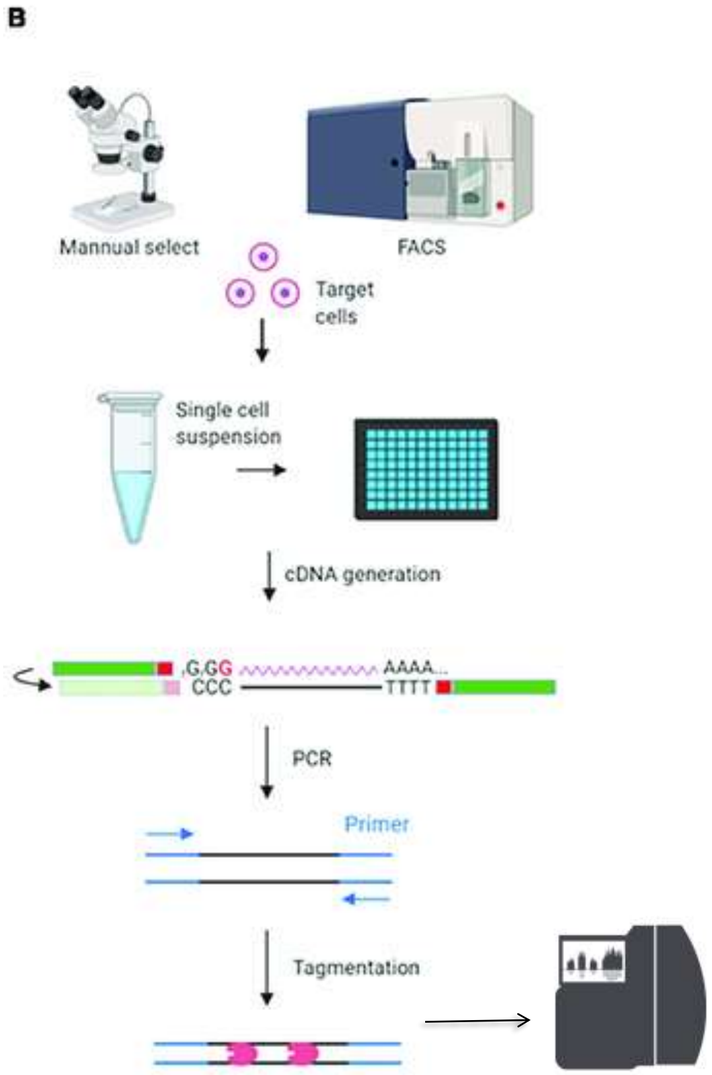
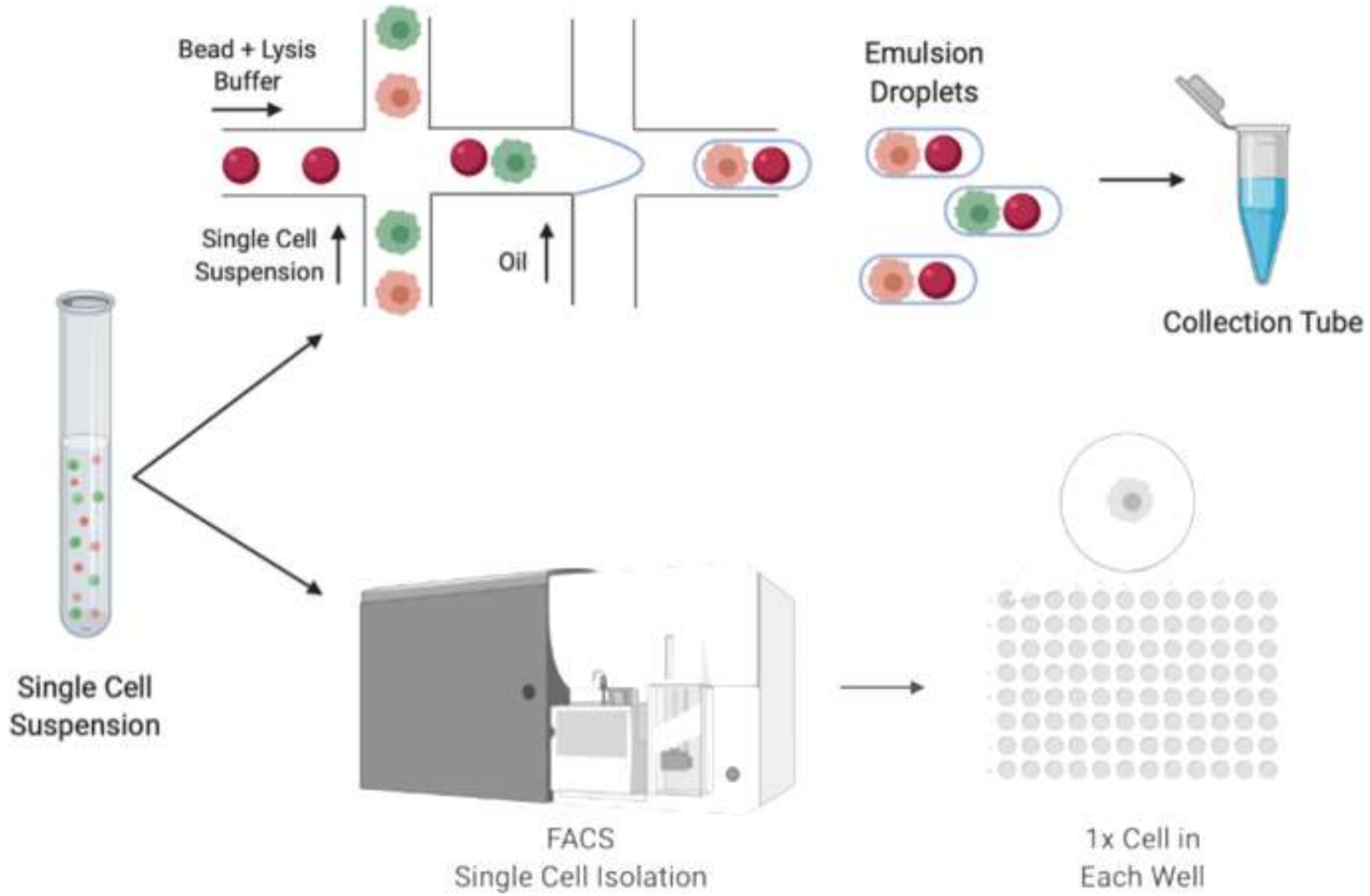

Bead + Lysis Buffer
Single Cell Suspension
Oil
Emulsion Droplets
Collection Tube
Single Cell Suspension
FACS Single Cell Isolation
1x Cell in Each Well

Plate-based


B
Mannual select
FACS
Target cells
Single cell suspension
cDNA generation
GGG
CCC
AAAA...
TTTT
PCR
Primer
Tagmentation

Protocols


Bead + Lysis Buffer
Single Cell Suspension
Oil
Emulsion Droplets
Collection Tube
Single Cell Suspension
FACS
Single Cell Isolation
1x Cell in Each Well

Droplet-based

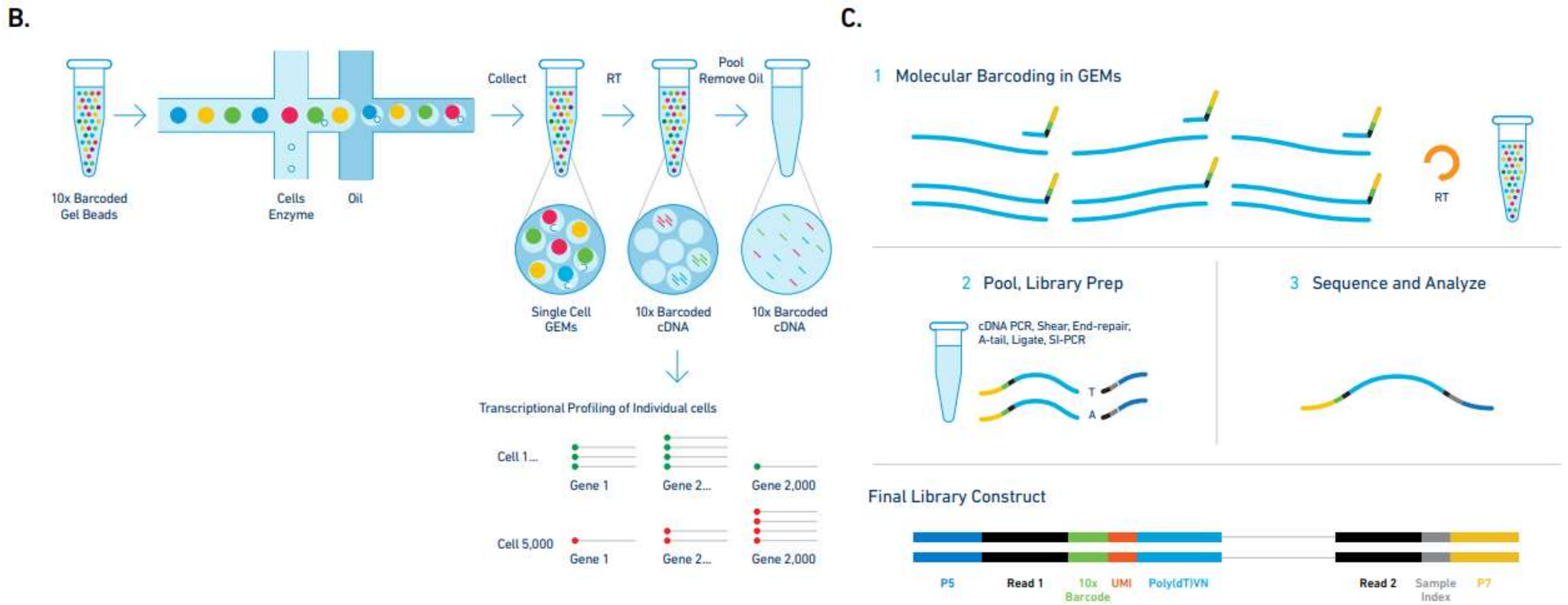


Figure 1. Chromium™ Single Cell 3′ Solution. (a) Workflow schematic overview. (b) Formation of GEMs, RT takes place inside each GEM, which is then pooled for cDNA amplification and library construction in bulk. (c) v2 Single Cell Assay schematic overview.

Thank you for you attention!

cnag

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centro nacional de análisis genómico

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